

---

# CLINICAL ASPECTS OF IMMUNOLOGY

EDITED BY

**P.J.LACHMANN**

ScD FRCP FRCPath FRS

*Sheila Joan Smith Professor of Immunology  
and Honorary Director of MRC Molecular Immunopathology Unit  
University of Cambridge, UK*

**SIR KEITH PETERS**

MD(Hon) MB FRCP FRCPath

*Regius Professor of Physic  
University of Cambridge School of Clinical Medicine  
Addenbrooke's Hospital, Cambridge, UK*

**F.S.ROSEN**

MD

*James L. Gamble Professor of Pediatrics  
Harvard Medical School, Boston, USA*

**M.J.WALPORT**

PhD FRCP MRCPPath

*Professor of Rheumatology  
Royal Postgraduate Medical School  
Hammersmith Hospital, London, UK*

IN THREE VOLUMES  
VOLUME 2

FIFTH EDITION

BOSTON

BLACKWELL SCIENTIFIC PUBLICATIONS

OXFORD LONDON EDINBURGH

MELBOURNE PARIS BERLIN VIENNA

© 1963, 1968, 1975, 1982, 1993 by  
Blackwell Scientific Publications, Inc.  
Editorial offices:  
238 Main Street, Cambridge  
Massachusetts 02142, USA  
Osney Mead, Oxford OX2 0EL, England  
25 John Street, London WC1N 2BL  
England  
23 Ainslie Place, Edinburgh EH3 6AJ  
Scotland  
54 University Street, Carlton  
Victoria 3053, Australia

Other Editorial Offices:  
Librairie Arnette SA  
2, rue Casimir-Delavigne  
75006 Paris  
France

Blackwell Wissenschafts-Verlag  
Meinekestrasse 4  
D-1000 Berlin 15  
Germany

Blackwell MZV  
Feldgasse 13  
A-1238 Wien  
Austria

All rights reserved. No part  
of this book may be reproduced  
in any form or by any electronic  
or mechanical means, including  
information storage and retrieval  
systems, without permission in  
writing from the publisher, except  
by a reviewer who may quote brief  
passages in a review.

First published 1963  
Revised reprint 1964  
Second edition 1968  
Third edition 1975  
Fourth edition 1982  
Fifth edition 1993

Set by Setrite Typesetters, Hong Kong  
Printed and bound in the USA  
by The Maple-Vail Book  
Manufacturing Group, New York

93 94 95 96 5 4 3 2 1

#### DISTRIBUTORS

##### USA

Blackwell Scientific Publications, Inc.  
238 Main Street  
Cambridge, Massachusetts 02142  
(Orders: Tel: 617 876-7000  
800 759-6102)

##### Canada

Times Mirror Professional Publishing, Ltd  
130 Flaska Drive  
Markham, Ontario L6G 1B8  
(Orders: Tel: 416 470-6739  
800 268-4178)

##### Australia

Blackwell Scientific Publications Pty Ltd  
54 University Street  
Carlton, Victoria 3053  
(Orders: Tel: 03 347-5552)

##### Outside North America and Australia

Marston Book Services Ltd  
PO Box 87  
Oxford OX2 0DT  
(Orders: Tel: 0865 791155  
Fax: 0865 791927  
Telex: 837515)

##### Library of Congress

##### Cataloguing-in-Publication Data

Clinical aspects of immunology/  
edited by P.J. Lachmann ... [et al.].  
—5th ed.

p. cm.

Includes bibliographical references and index.

ISBN 0-86542-297-4

1. Clinical immunology. 2. Immunology.

I. Lachmann, P.F. (Peter Julius)

[DNLM: 1. Hypersensitivity. 2. Immunity.

WD 300 C641]

RC582.C52 1993

616.07'9—dc20

DNLM/DLC

for Library of Congress

new class of recombinant antibody fragments and may be of use in therapy and diagnostics. In addition, it is expected that they could be used as building-blocks for Fv and Fab fragments or complete antibodies. The small size of the Fv fragment and single-domain antibody should facilitate the determination of the three-dimensional structure of the antigen-binding site by both X-ray crystallography and nuclear magnetic resonance (NMR) analyses.

Fab fragments have been secreted from *E. coli* (Better *et al.* 1988). (Fab)<sub>2</sub>' fragments (Neuberger *et al.* 1984) and a Fab-like fragment, with V<sub>H</sub> and V<sub>L</sub> domains each joined to C<sub>k</sub> domains (Sharon *et al.* 1984), have been secreted from myeloma cells. Also a range of fragments containing parts of the Fc region of IgE have been expressed in *E. coli* as inclusion bodies and refolded. This expression system has been used to localize the binding site for the IgE receptor, Fc<sub>ε</sub>RI, on the Cε2 and Cε3 domains (Helm *et al.* 1988).

#### **Antibody fragments joined to other proteins: enzymes, toxins and receptors**

At its simplest, making a gene fusion with antibody may provide a handle for its detection and purification (Neuberger *et al.* 1984). Furthermore, enzyme activities, such as alkaline phosphatase or horse-radish peroxidase linked directly to Fab fragments could be invaluable for enzyme-linked immunosorbent assay (ELISA) and other diagnostic tests. Genes encoding staphylococcal nuclease and Klenow polymerase (Neuberger *et al.* 1984) were linked to antibody Fab genes as a model system for such antibody fusions. The enzymes, which are monomeric, were attached to the N-terminal portion of the IgG C<sub>H</sub>2 domain. This region of the antibody, which leads from the hinge, is disordered in the crystallographic structure, and may provide a flexible link. Both Fab-enzyme and (Fab)<sub>2</sub>'-enzyme constructs were expressed (the hinge sulph-hydryl groups are present), and the constructs shown to possess both antigen-binding activity and enzyme activity.

Fab-enzyme fusions may also have therapeutic potential, although the enzyme moiety is likely to be immunogenic. For example, tissue plasminogen activator (TPA) has been linked to an anti-fibrin Fab, which should direct the TPA to the vicinity of blood clots, where it can locally activate plasmin-

ogen (Schnee *et al.* 1987). Likewise for pro-drug therapy, an enzyme capable of cleaving the pro-drug could be linked to a suitable Fab or (Fab)<sub>2</sub>' fragment. Yet another possibility lies in linking a toxin, such as ricin, diphtheria toxin or *Pseudomonas* exotoxin, to the Fab or Fv fragment, to create an immunotoxin (Chaudhary *et al.* 1989; for review, see Ahmad and Law 1988). Here the strategy is different: such toxins must be delivered inside the target cell, where, for example, they catalyse the ribosylation of EFTu. A single molecule of toxin can kill the cell. However the entry of the Fab/Fv-toxin into the target cells is likely to prove difficult, as well as its expression and secretion from myeloma cells.

Other kinds of antibody fusions have been made, for example in which the antibody variable domains have been replaced by a T cell receptor V<sub>α</sub> domain, on either the Ig heavy chain (Gascoigne *et al.* 1987) or the Ig light chain (Mariuzza and Winter 1989) constant domains. Likewise CD4, which binds to the acquired immune deficiency syndrome (AIDS) human immunodeficiency virus (HIV)-1 gp(glycoprotein)120 coat protein, has been assembled with Ig heavy chain constant regions to create an 'immuno-adhesin'. The fusion enhances the serum half-life of CD4 and the antibody effector functions may prove effective in killing AIDS virus-infected cells (Capon *et al.* 1989).

#### **Simple chimeric antibodies**

During the maturation of the immune response, the class of Ig heavy chain is switched from IgM to IgG (for review see Shimizu and Honjo 1984). Likewise the isotype of IgG can be switched and involves bringing the rearranged V<sub>H</sub> gene into the proximity of a new set of constant region genes, with deletion of the intervening DNA (Davis *et al.* 1980; Maki *et al.* 1980; Kataoka *et al.* 1981; Obata *et al.* 1981). As expected from the Ig domain structure, class switching does not seem to alter the affinity of the antibody for antigen (Neuberger and Rajewsky 1981). Class switching underlies the concept of simple chimeric antibodies: the variable regions from one source are attached to constant regions from another. This allows the species, class and isotype of constant regions to be selected for the antibody, which in turn dictates its immunogenicity and effector functions.

A variety of simple chimeric antibodies have been made (Boulianne *et al.* 1987; Morrison *et al.* 1984; Neuberger *et al.* 1985; Sun *et al.* 1987). The most extensive collection is based on the mouse B1-8 heavy chain with mouse  $\lambda$  light chain (Brüggeman *et al.* 1987). The heavy chain variable region has been attached to a variety of mouse, rat and human constant regions. These antibodies have a specificity for the haptens NP (and NIP (Neuberger and Rajewsky 1981)), and are readily purified on columns of hapten-Sepharose. Cell surfaces are readily derivatized using NIP-succinimide ester (which reacts with protein cell surface markers) or NIP-cephalin (which inserts into the membrane lipid), and this facilitates the assay of effector functions such as complement lysis and cell-mediated killing (for reviews see Winkelhake *et al.* 1978; Burton 1987). Such 'matched sets' of chimeric antibodies have proved invaluable for comparing effector functions (Brüggeman *et al.* 1987; Stepelewski *et al.* 1988) and segmental flexibility (Oi *et al.* 1984; Dangel *et al.* 1988) of different antibody isotypes. For example, it emerges that the human  $\gamma 1$  isotype is the most active in complement lysis and ADCC, indicating that it is the most suitable for therapy (Brüggeman *et al.* 1987; Riechmann *et al.* 1988a).

### Reshaped antibodies

The antigen-binding site is localized to the loops at the tips of the variable region, and correlates with the regions of hypervariable sequence (or complementarity-determining regions, CDRs) in each of the  $V_H$  and  $V_L$  domains (Kabat *et al.* 1987). Reshaped antibodies, in which the antigen-binding site only is derived from another antibody, have been constructed by transplanting the CDRs (Jones *et al.* 1986; Riechmann *et al.* 1988a; Verhoeyen *et al.* 1988). This not only allows the choice of constant region, as with the simple chimeric antibodies, but also the choice of variable region  $\beta$ -sheet 'framework' and loops adjacent to the constant domains. Such human antibodies, in which only the antigen-binding site is derived from a mouse antibody, may prove less immunogenic in humans than a simple chimeric antibody, in which the entire variable region is taken from a mouse antibody. However, there are several assumptions underlying this approach. In particular, certain structural features of both antibodies

must be matched for the graft to 'take' on its new framework: the packing of the heavy and light chain variable domains; the packing of the two  $\beta$ -sheets within a domain; the packing of the CDRs on to the  $\beta$ -sheet framework; and the contact of the antigen only with CDR residues (or conserved residues in the  $\beta$ -sheet). Inspection of the available crystallographic structures suggests that the packing of domains, sheets and loops are relatively conserved (Lesk and Chothia 1982; Chothia and Lesk 1987) and that the vast majority of contacts between antibody and antigen are made via CDR residues (Amit *et al.* 1986; Sheriff *et al.* 1987; Padlan *et al.* 1989). Nevertheless, in reshaping the anti-lymphocyte antibody, CAMPATH-1, to avoid loss of binding activity the framework region of the human antibody had to be adapted to accommodate the CDRs of the rat antibody (Riechmann *et al.* 1988a).

### Dissecting antibody affinity and effector functions

The diversity of antibody-binding sites is not only based on the recombinatorial diversity of the genetic elements ( $V_H$ , D,  $J_H$ ;  $V_K$ ,  $J_K$ ;  $V_L$ ,  $J_L$ ) and diversity at the junctions, but also on somatic mutation during the maturation of the immune response (for review see Tonegawa 1983). Point mutations in antibody variable regions are sufficient to alter antibody affinity and specificity (Rudikoff *et al.* 1982; Roberts *et al.* 1987). For example, higher-affinity antibodies derived from a  $V_H$  gene family which expresses antibodies directed against the hapten NP appear to be determined by a replacement of tryptophan 33 by leucine (Allen *et al.* 1988). It should also be possible to construct higher-affinity antibodies by design: mutations constructed in the hypervariable regions of an anti-lysozyme antibody were shown to increase its affinity for antigen (Roberts *et al.* 1987). Enhancing the affinity and specificity of antibodies has important practical applications, for example in increasing the sensitivity of ELISA or of *in vivo* imaging and therapy.

The binding of antibody to a cell receptor has been illuminated by construction of a single mutant. Binding of antigen-antibody complexes to specialized cells via three receptors, FcRI, FcRII and FcRIII (Anderson and Looney 1986), can trigger ADCC. The high-affinity receptor FcRI appears